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ARMSTRONG LABORATORY

EFFECT OF INTERMITTENT COLD EXPOSURE ON THE FIBER-TYPE COMPOSITION OF SELECTED SKELETAL MUSCLES IN RATS

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources—National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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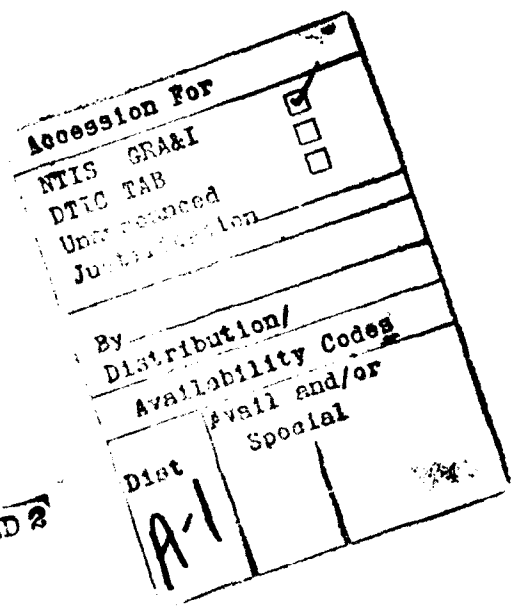

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We examined the effect of long-term intermittent cold exposure (CE) on the fiber-type composition of the predominantly type I soleus and the predominantly type IIb extensor digitorum longus (EDL) muscles of the rats. CE was accomplished by submerging the rats in shoulder-deep water, maintained at 20 ± 0.5 °C, for 1 h/d, 5 d/wk, for up to 19 weeks. Rats were randomly assigned to either a Control (CON) or Cold Exposure group. The efficacy of the treatment was tested by subjecting both groups to 20 °C water for 45 minutes while measuring rectal temperature (T_{re}) and VO_2. The CE group displayed a 22% smaller reduction in T_{re} ($p < 0.05$) at the end of the exposure, and had a 23% greater VO_2 ($p < 0.05$) during the same period. Fiber-type composition was determined using routine histochemical methods for myosin-ATPase. The soleus muscle of the CE rats underwent a 156% increase in the number of type IIa fibers ($p < 0.05$), with a 24% reduction in type I fibers ($p < 0.05$). CE had no significant influence on the fiber-type composition of the EDL muscle. CE resulted in an increase in citrate synthase activity of 20% and 22% in the soleus and EDL muscles, respectively ($p < 0.05$). The present study demonstrates that intermittent CE induces a type I-to-type IIa transformation in the soleus muscle while having no influence on the EDL muscle.

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INTRODUCTION

Muscle function is affected by temperature (4). As a result of the Q_{10} effect, a decline in muscle temperature results in a reduction in the rate of flux through enzyme systems, which include myosin-ATPase (M-ATPase) and the mitochondrial enzymes involved in ATP production. A reduction in M-ATPase activity results in a slower speed of muscle contraction, while the reduction in mitochondrial enzyme activity results in a reduced rate of ATP production (4) In poikilotherms, such as many species of fish and lizards, acclimation to seasonal declines in temperature results in an increase in M-ATPase activity (5,15,16), as well as an increase in mitochondrial enzyme activity (5,14). These adaptations partially compensate for the temperature-induced decline in muscle function.

Although increases in skeletal muscle mitochondrial enzyme activity (8,9) and M-ATPase activity (3) have been observed in cold-acclimated mammals, alterations in fiber-type composition have not. The fact that there are no reports of fiber-type composition in cold-acclimated mammals may be due to the fact that the typical method for inducing cold acclimation in mammals is exposure to cold air (1,8,9). Water has a much greater heat capacity than air and therefore results in a much greater loss of heat, thus possibly providing a greater stimulus intensity for the induction of a fiber-type shift.

We tested the hypothesis that a shift in fiber-type composition would occur in rats if they were given an adequate cold stimulus. Rats were exposed daily (1 h) to cold water (20 °C) over a 17- to 19-wk period. Additionally, in order to indirectly compare this method of cold exposure with traditional methods involving cold air, skeletal muscle oxidative enzyme activity and whole-body metabolic profiles were also examined.

METHODS

Twenty male rats (Sprague-Dawley CD-VAF/Plus), initially 353 ± 28 g, were obtained from the colonies of Charles River Laboratories (Wilmington, MA). Rats were multiply housed, 4 rats to a cage, until they reached body weights exceeding 500 g, at which point they were housed 2 rats per cage. Rats were allowed *ad libitum* access to food (Purina Rodent Chow) and water. The room in which the rats were kept was maintained on a 0600-1800 light cycle at 25 ± 1 °C.

Experimental Treatment

Rats were randomly assigned to either a control (CON) or cold exposure (CE) group. Cold exposure was accomplished by submerging the rats in shoulder-deep water in a 50-gallon container, 5 rats/container. The water temperature was constantly monitored and was maintained at 20.0 ± 0.5 °C by the periodic addition of ice cubes. Initially, the rats were exposed for 5 min/d, with an additional 5 min added each day until a final period of 60 min/d was reached. The CE rats received this treatment 5 d/wk for 17 to 19 wk.

Efficacy of Treatment

To evaluate the efficacy of the cold exposure treatment, CON (n=5) and CE (n=5) rats were cold exposed while monitoring rectal temperature with a Vitec thermal probe inserted 5 cm into the rectum (Figure 1). In addition, the oxygen consumption (V_{O_2}) during the cold exposure was determined (Figure 2). Due to the drastic decline in the T_{re} of the CON rats, the exposure was terminated after 45 min in this group. The exposure involved placing a rat into a cylindrical metabolic chamber (750 cm^3) which was in turn placed in the water as previously described. The metabolic chamber had holes up to a point corresponding to 1 cm above the waterline. The holes below the waterline allowed for the mixing of water between the chamber and the 50-gal container, while the holes above the waterline allowed the entry of air, which was pulled through the chamber and exited through a mixing chamber at the top. Exhaust was pulled by at a rate of 600 ml/min, and FeO_2 and $FeCO_2$ were determined in line with a Perkin-Elmer 11000 Medical Gas Analyzer. The V_{O_2} was computed by a Macintosh II computer interfaced with the gas analyzer and flow meter, using Lab View™ data acquisition/analysis software package, as previously described (2). The rats on which these data were determined were familiarized with the procedure by periodically placing them in the metabolic chamber and placing it into the water prior to the actual experiment.

Food Intake

The daily food intake of the rats was estimated by determining the amount of food consumed/cage for the last week of the study. This value was divided by 2 (2 rats/cage), and then divided by 7 (7 d/wk).

Histochemical Analysis

Rats were euthanized with an overdose of Nembutal. The soleus and extensor digitorum longus (EDL) muscles were excised, weighed, and pinned to a wooden stick. The muscles were then frozen in isopentane cooled (-100 °C) in liquid N_2. Cryostat sections (8μ) were stained for myosin-ATPase using routine histochemical methods (7) at pH's of 4.53 and 4.30. The fiber type of each muscle was determined from 3 photomicrographs/muscle. This represented 250-350 fibers/photomicrograph. The fibers were classified according to the nomenclature of Staron and Pette (20).

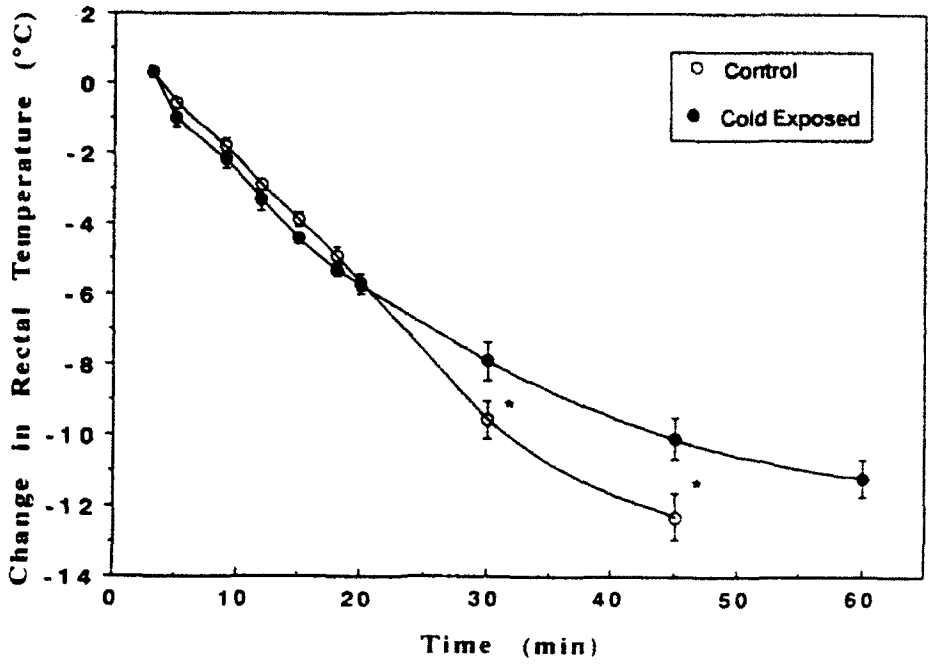

Figure 1.
The influence of cold water immersion (20 °C) on the T_{re} of CON and CE rats. The pre-, i.e., time = 0, T_{re} was 37.2 ± 0.1 °C for the CON and CE groups, respectively. *Significantly different from CON ($p < 0.05$).

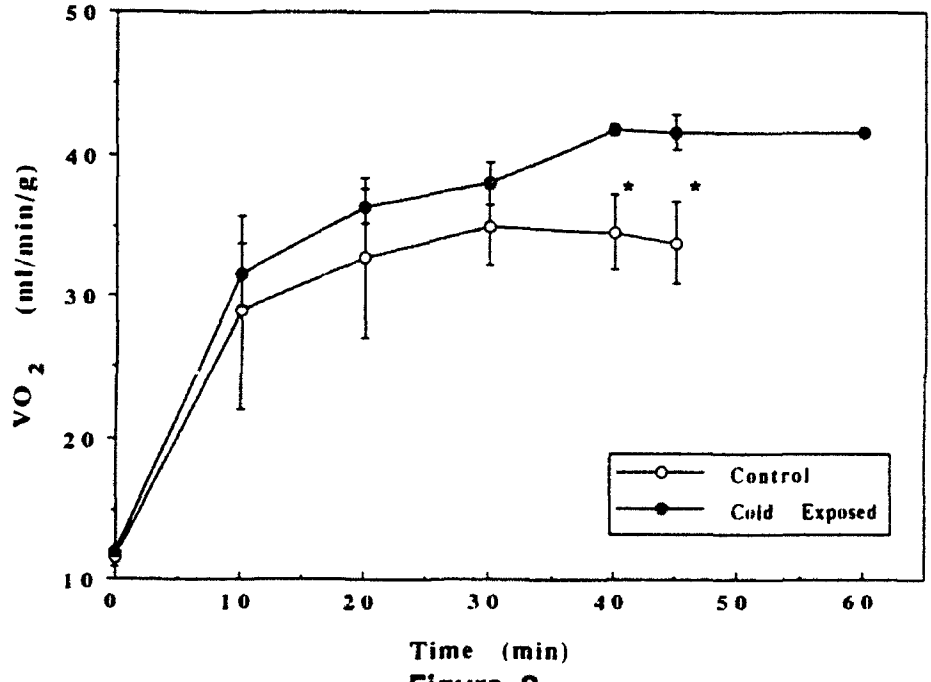

Figure 2.
The influence of cold water immersion (20 °C) on the V_{O_2} of CON and CE rats. *Significantly different from CO ($p < 0.05$).

Citrate Synthase Activity

Citrate synthase activity was determined in muscle homogenates by the spectrophotometric method of Srere (19).

Statistics

Significance between groups was determined using a t-test. The level of significance was preset at $p = 0.05$.

RESULTS

Animal Weights and Food Intake

The CE rats weighed 6% less than the CON rats ($p < 0.05$) (Table 1), while they consumed 37% more food (g food/day/g of body wt.) compared to the CON rats ($p < 0.05$) (Table 1).

Table 1. Body Weights and Food Intake of Control and Cold-Exposed Rats

Group	Body Weight (g)	Daily Food Intake (g food/day/g body weight)
Control (n=10)	630 ±10	35.02 ±1.86
Cold Exposed (n=10)	594* ± 9	47.53* ±1.14

All rats had ad libitum access to food (Purina Rodent Chow).
Values expressed are mean ±SEM.
*Significantly different from Control values ($p < 0.05$).

Efficacy of the Cold Exposure Treatment

The severity of the cold exposure treatment used in the present study can be seen in Figure 1. Both CON and CE rats underwent a significant reduction in T_{re} in response to the treatment. However, both the rate and amount of reduction in T_{re} were significantly less in the CE group during the exposure. The ability of the CE group to maintain a higher T_{re} was accompanied by a significantly greater metabolic rate during the exposure (Figure 2). The relative

difference of the VO_2's and T_{re} between the CE and CON rats was 22% and 23%, respectively.

Fiber-Type Composition

The cold exposure had a significant impact on the fiber-type composition of the soleus muscle. The soleus of the CE rats underwent a three-fold increase in the number of type IIa fibers (12.1 ± 0.3% vs. 31.0 ± 3.3%) ($p < 0.05$), with a 24% reduction in type I fibers (80.9 ± 3.0% vs. 64.7 ± 3.9%) ($p < 0.05$) (Table 2). CE had no significant influence on the fiber-type composition of the EDL (Table 2).

Table 2. Fiber-type Composition of the Soleus and EDL Muscles from Control and Cold-Exposed Rats

Group	Muscle	Type I (%)	Type Ic (%)	Type IIa (%)	Type IIb (%)
CON	Soleus	80.9	7.0	12.1	—
(n=5)		±3.0	±3.1	±0.3	—
CE		64.7*	4.3	31.0*	—
(n=5)		±3.9	±1.6	±3.3	—
CON	EDL	2.9	—	23.6	76.4
(n=5)		±0.6	—	±0.9	±0.9
CE		3.8	—	25.7	71.0
(n=5)		±1.0	—	±6.1	±6.1

All values expressed are means ± SEM.
*Significantly different from Control ($p < 0.05$).

Citrate Synthase Activity

Cold exposure resulted in a 22% increase ($p < 0.05$) in citrate synthase activity in the soleus muscle. The EDL muscle of the CE rats was 23% greater than that of the CON rats ($p < 0.05$) (Table 3).

Table 3. Citrate Synthase Activity of the Soleus and EDL Muscles from Control and Cold-Exposed Rats

Group	Soleus (μmol/min/g)	EDL (μmol/min/g)
Control (n=5)	27.0 ±1.2	22.3 ±0.6
Cold Exposed (n=5)	32.9* ±0.9	27.4* ±1.1

All values expressed are mean ± SEM.
*Significantly different from Control ($p < 0.05$).

DISCUSSION

The cold exposure treatment used in the present study clearly resulted in a number of adaptive responses. The increase in oxidative enzyme activity that occurred in the soleus and EDL muscles of the rats in the present study is consistent with a number of earlier reports for both mammals (8,9) and poikilotherms (6,14). Temperature has a profound influence on skeletal muscle by reducing the rate of metabolic flux as temperature declines. An increase in oxidative enzyme activity thus serves as a positive adaptation to cold by which the aerobic capacity of the muscles can be maintained.

The present study demonstrates that sufficient intermittent cold exposure can induce a fiber-type shift in predominantly type I fibers in rats. This finding is consistent with reports of decreased M-ATPase activity in muscle homogenates of cold-adapted fish (11,15) and rats (3). As the temperature of skeletal muscle declines, so does its contractile speed (4,17,18). In the soleus muscle the shift in fiber type from slow contracting type I fibers to an increasing percentage of faster contracting type IIa would compensate for the temperature-induced reduction in contractile speed. Consistent with this reasoning is the lack of a shift in the predominantly type IIb EDL muscle; i.e., it is already predominantly composed of the fastest contracting muscle fibers.

The cold exposure treatment used in the present study would be expected to result in elevated circulating thyroid hormone (TH) levels (1,12). A recent study, examining the influence of cold exposure on myosin heavy chain (MHC) expression in cardiac muscle, demonstrated an increase in the proportion of the ∂-MHC with a concomitant increase in circulating TH levels (1). Furthermore, Izumo et al. (13) have shown that elevated TH levels in rats results in an increase in the expression of the IIa myosin heavy chain in the soleus muscle, while having little effect on the EDL muscle. Finally, TH is a potent stimulus for increased oxidative enzyme activity (21). Taken together these data provide indirect support for the possible role of TH in mediating the alterations reported in the soleus and EDL muscles following cold exposure in the present study.

Alternatively, Hazel and Prosser (10) have proposed that cold exposure alone may induce the expression of temperature-specific isozyme genes. If this is true, cold water may be more effective than cold air in inducing a fiber-type shift because the greater heat capacity of water would be expected to result in a greater reduction in local muscle temperature than would occur with cold air. Furthermore, the increase in oxidative enzyme activity reported here is greater than that reported for rats chronically maintained in cold air (5 °C) (9).

The CE treatment clearly resulted in adaptations which were manifested in a slower rate of temperature decline in the CE rats (Figure 1). Although not measured in the present study, the significantly greater VO_2 displayed by the CE rats in Figure 2 likely reflects an adaptive increase in the mass and metabolic activity of brown adipose tissue (9). Additionally, the significantly greater food intake in the CE rats represents another important manifestation of cold adaptation (9).

In conclusion, long-term, intermittent cold exposure using cold water (20 °C) for 1 h/d induced a significant type I-to-type II fiber shift in the soleus muscle, as well as significant increases in oxidative enzyme activity in the soleus and EDL muscles of rats. The metabolic adaptations induced by this treatment mode are comparable to those reported for chronic cold air exposure.

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